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EP-A-0 025 889
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CLINICAL CHEMISTRY, vol. 29, no. 1, January 1983, pages 37-41, Washington, US; G.M. DAPPEN et al.: "A diazo-based dry film for determination of total bilirubin in serum"

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Description

The present invention relates to an analytical element for quantitative analysis of bilirubin in a liquid sample by diazo method comprising a liquid impermeable, light-transmittive support, a reagent layer containing a bilirubin detection reagent and a liquid sample-spreading layer.

Bilirubin, a principal component of the bile pigment in a body fluid, is produced in serum by decomposition of heme originating from hemoglobin in red blood corpuscle. Bilirubin is then absorbed by the liver, in which bilirubin is converted to a glucuronic acid-conjugated product, and excreted in bile. The content of bilirubin in blood increases in response to increase of decomposition of hemoglobin as well as decrease of the liver function. Accordingly, the quantitative analysis of bilirubin is considered to be an indispensible test item in the clinical test.

As the method for quantitative analysis of bilirubin in serum, there are known a quantitative analysis method comprising photometric measurement of the yellow color inherently attached to the bilirubin, and a colorimetric analysis of red azobilirubin produced by coupling reaction of bilirubin and diazotized sulfanilate (p-sulfobenzenediazonium salt, Ehrlich reagent) based on Ehrlich reaction discovered by Van den Bergh. The latter method is named a diazo method.

Details of methods for quantitative analysis of bilirubin in serum are described in "Comprehensive Text of Clinical Test Technology" edited by Ishii, Vol. 6, pp. 332—350 (Igaku Shoin, 1975).

Details of the diazo method are further described below.

Bilirubin produced in serum by the decomposition of heme is named free bilirubin. This bilirubin is as such hydrophobic, but is dissolved in serum in combination with serum albumin, being adsorbed by the serum albumin. The free bilirubin introduced into liver is combined with glucuronic acid through covalent bond to become conjugated with glucuronic acid. Thus, a glucuronic acid-conjugated bilirubin which is enhanced in the water-solubility by the aid of the hydrophilic group contained in the glucuronic acid is produced. Also known is a highly water-soluble bilirubin combined to serum albumin, but no production mechanism is known for this product (J. J. Lauff, et al., Clinical Chemistry, *28*(4), 629—637 (1982)).

Among these various bilirubins, the highly water-soluble conjugated bilirubin and the albumin-conjugate bilirubin both easily react with a diazonium salt, and are directly subjected to colorimetry. Accordingly, these bilirubins are named direct bilirubins.

The hydrophobic free bilirubin undergoes coupling reaction in the presence of a reaction accelerator such as caffeine, sodium benzoate, sodium acetate, dyphylline (C. A. Registry No. [479—18—5], urea, a nonionic surfactant, gum arabic, an alcohol (e.g., methanol, ethanol), an acid amide or sulfoxide, to produce azobilirubin. Therefore, the quantitative analysis of free bilirubin is generally performed indirectly by a stage of colorimetrically determining the total bilirubin content in a liquid sample in the presence of a reaction accelerator and a subsequent stage of subtracting the direct bilirubin content determined separately in the absence of a reaction accelerator from the total bilirubin content. For this reason, the free bilirubin is otherwise named an indirect bilirubin.

Details of the diazo method for quantitative analysis of bilirubin are described in the following publications: M. Michaelsson, Scand. J. Clin. Lab. Invest., *13* (Suppl.), 1—80 (1961); H. Malloy, J. Biol. Chem., *119*, 481(1939); and Z. K. Shihabi, et al., American Journal of Medical Technology, 43(10), 1004—1007 (1977).

As for the diazonium salt employed in the bilirubin analysis based on the diazo method, improvements have been recently made with respect to the detection sensitivity and stability of the produced azobilirubin. For instance, halobenzenediazonium salts such as 2,4-dichlorophenyldiazonium salt and 2-chloro-4-nitrophenyldiazonium, and stabilized diazonium salts (stabilized by the use of counter ions) developed by Kulhanek, Erthinghansen, et al. are generally utilized. The history of such development is described in Japanese Patent Publication No. 54(1979)—12840, and Japanese Patent Provisional Publications Nos. 55(1980)—4492, 56(1981)—10255, 56(1981)—12555 and 57(1982)—103056.

GB—A—2 085 581 discloses a method of bilirubin detection and a device therefore. A method of quantitative determination of bilirubin comprises bringing a bilirubin-containing aqueous liquid sample into contact with a hydrophobic bilirubin extracting composition containing a hydrophobic amine capable of extracting bilirubin, and using photometry to determine the concentration of bilirubin extracted. In a test strip device for use in this method one layer is impregnated with the hydrophobic bilirubin extracting composition. The hydrophobic amine may be dodecenyl (trialkylmethyl) amine or di-cyclohexylamine. Multilayer devices are described.

As described above, a colorimetric analysis method comprising performing a color reaction in proportion to the content of an analyte (substance to be analyzed) and subsequently measuring the color formation to determine the content of the analyte is well known. This method is utilized not only in a wet analysis process but also in a dry analysis process.

The dry process (i.e., dry analysis process) is based on a colorimetric analysis utilizing a dry analytical element in the form similar to the pH test strip, which comprises a paper sheet or absorbent carrier impregnated with a reagent to produce a color in contact with an analyte.

As the dry analytical element, there is known a multilayer analytical element capable of giving highly precise analytical results. For instance, multilayer analytical elements, described in Japanese Patent Publication 53(1978)—21677 (corresponding to US—A—(3,992,158), and Japanese Patent Provisional

Publications Nos. 50(1975)—137192 (US—A—(3,983,005), 51(1976)—40191 (US—A—4,042,335), 52(1977)—3488 (US—A—(Re 30,267), 53(1978)—89796 (US—A—(4,069,017) and 53(1978)—131089 (US—A—(4,144,306), are in the form of a laminated structure comprising a support, one or more reagent layers on the support, and a porous, nonfibrous spreading layer on the reagent layer.

The above-mentioned multilayer analytical element is constructed in such a manner that a liquid sample applied (for instance, spotted) onto the spreading layer permeates into the reagent layer, keeping a substantially constant amount per unit area, and shows therein a color reaction. Accordingly, the content of the analyte in the liquid sample can be determined by measuring the color density after the lapse of a certain period of time.

GB—A—2 085 159 discloses a multilayer element for use in quantitative chemical analysis. A multilayer chemical analysis element comprises: a light-transmissible, water-impermeable support; a first reagent layer provided on said support and comprising a color-forming reaction layer comprised of a chromogen compound capable of forming a dye upon reaction with a dye-forming reactive group; and a second reagent layer comprising a substrate layer comprised of a non-diffusible substrate comprising said dye-forming reactive group which is substantially colorless and is cabable of forming a dye upon reaction with said chromogen compound, the substrates being capable of reacting with an analyte to form a substantially colorless diffusible compound which contains the dye-forming reactive group. The element may include further functional layers. An element for the quantitative determination of amylase activity in blood or other living body fluids is described.

A multilayer analytical element for quantitative analysis of bilirubin based on the dry process is already known. This element utilizes a color reaction between bilirubin and diazotized sulfanilate (p-sulfobenzenediazonium, a bilirubin detection reagent) in the reagent layer thereof.

However, since the above-mentioned diazonium salt is highly hydrophilic and of high polarity, some problems are brought about in the analytical process employing the multilayer analytical element. For example, in the course of diffusion of the liquid sample into the reagent layer after spotting the liquid sample on the spreading layer of the analytical element, the diazonium salt is liable to be distributed un-uniformly through the so-called chromatographic behavior to reduce the uniform distribution of the azobilirubin showing color.

Moreover, the diazonium salt is liable to diffuse between the layers in the course of the preparation and storage of the multilayer analytical element, whereby the accuracy of the bilirubin analysis decreases as the time progresses. This means that the effectively employable period of the analytical element is shortened.

Into the multilayer analytical element, certain improvements have been introduced for enhancing the accuracy of the measurement. For instance, a light-blocking layer and an isotropically porous spreading layer are provided to the element. Otherwise, the diazonium salt is locally located in the analytical element. Even in thus improved multilayer analytical elements, the above-mentioned low molecular weight diazonium salt contained in the reagent layer is very liable to diffuse into the light-blocking layer and spreading layer, in the course of the preparation and storage thereof. The high diffusive property of this diazonium salt is considered to arise from its low molecular weight. In the multilayer analytical element containing thus diffused diazonium salt, bilirubin contained in a liquid sample spotted thereon produces a not a small amount of azobilirubin even within the light-blocking layer and spreading layer. Accordingly, the total bilirubin produced by bilirubin and the diazonium salt cannot be quantitatively measured by a reflection optical measurement. In other words, the measured value shows a negative error.

A primary object of the present invention is to provide an analytical element employable for quantitatively analyzing bilirubin in a liquid sample, simply, quickly and very accurately.

Another object of the invention is to provide an analytical element containing a nondiffusive diazonium salt for quantitative analysis of bilirubin which is so improved that the diffusion of diazonium salt occurring in the course of the preparation and storage of the element is effectively prevented, whereby the error in measurement is remarkably reduced.

The present invention provides an analytical element for quantitative analysis of bilirubin in a liquid sample by diazo method comprising a liquid impermeable, light-transmissive support, a reagent layer containing a bilirubin detection reagent and a light sample-spreading layer, which is characterized in that the reagent layer is a porous reagent layer having continuous voids and the bilirubin detection reagent is a non-diffusive aryl diazonium salt having in the aryl group at least one substituent selected from an alkoxycarbonyl group, an alkylaminosulfonyl group and an alkylaminocarbonyl group.

Figure 1 shows graphically a relationship between the total bilirubin content and the optical density of the color formed on the analytical element, obtained in the analysis of bilirubin using an analytical element described in Example 1.

Figure 2 shows absorbtion spectra of azobilirubins produced in the use of a compound belonging to the aryl diazonium salt defined in the present invention as well as in the use of the conventional diazotized sulfanilate (p-sulfobenzenediazonium salt).

A representative embodiment of the analytical element of the present invention is in the form of a multilayer analytical element comprising a liquid sample-spreading layer (or simply spreading layer), one or more reagent layers, and a liquid-impermeable, light-transmissive support.

In the above-mentioned constitution, the support and liquid sample-spreading layer are known in their materials and constitutions. Accordingly, the support and liquid sample-spreading layer employable for

constituting the multilayer analytical element according to the invention can be optionally formed utilizing these known materials and constitutions. If desired, one or more of functional layers known in the structures of the conventional multilayer analytical elements, for instance, a light-reflective layer, a light-blocking layer, a diffusion-preventing layer, and an adhesive layer (to be attached to the reagent layer) can be provided in the element.

If the laminated structure comprising the reagent layer and liquid sample-spreading layer is in the form of a self-supporting integrated sheet, the reagent layer and/or the liquid sample-spreading layer as such can serve as a support replacing the independent support. The liquid sample-spreading layer is not essential to the constitution of the analytical element of the present invention.

The analytical element of the present invention contains, as a bilirubin detection reagent, a nondiffusive (diffusion-resistant) aryldiazonium salt having a specific substituent or substituents.

The aryldiazonium salt utilizable in the present invention contains in the aryl group (preferably a benzene ring) at least one substituent selected from an alkoxycarbonyl group, an alkylaminosulfonyl group and an alkylaminocarbonyl group (in which each of the alkyl and alkoxy preferably contains 2—22 carbon atoms), and preferably further contains at least one substituent selected from an alkyl group and an alkoxy group (in which each of alkyl and alkoxy preferably contains 1—12 carbon atoms).

Representative examples of the aryldiazonium salt utilizable in the present invention are given below, but these examples are by no means construed to restrict the invention:

1: 2-methoxy-5-(tetradecyloxycarbonyl)benzenediazonium tetrafluoroborate,
2: 2-methoxy-5-(tetradecyloxycarbonyl)benzenediazonium hexafluoroborate,
3: 2-ethoxy-5-(hexadecyloxycarbonyl)benzenediazonium tetrafluoroborate,
4: 2-dodecyloxy-5-(ethoxycarbonyl)benzenediazonium tetrafluoroborate,
5: 2-methoxy-5-[ß-(2',4'-di-t-amylphenoxy)ethoxycarbonyl]benzenediazonium tetrafluoroborate,
6: 2-methoxy-5-(N-hexadecylsulfamoyl)benzenediazonium tetrafluoroborate,
7: 2-propoxy-5-(N-tetradecylsulfamoyl)benzenediazonium perchlorate,
8: 2-octyloxy-5-(N-decylsulfamoyl)benzenediazonium hexafluorophosphate,
9: 3,5-bis(dodecyloxycarbonyl)benzenediazonium tetrafluoroborate,
10: 3,5-bis(tetradecyloxycarbonyl)benzenediazonium tetrafluoroborate,
11: 2-methoxy-5-(N-tetradecylcarbomoyl)benzenediazonium tosylate,
12: 2-methoxy-5-[N-(4-t-amylphenoxyethyl)carbamoyl]benzenediazonium 1-naphthalenesulfonate,
13: 4-(hexadecyloxycarbonyl)benzenediazonium tetrafluoroborate,
14: 4-(N-hexadecylsulfamoyl)benzenediazonium tetrafluoroborate,
15: 3-hexadecylcarbonylbenzenediazonium tetrafluoroborate,
16: 3-(N-tetradecylcarbamoyl)benzenediazonium tetrafluoroborate,
17: 2-methyl-5-tetradecyloxycarbonylbenzenediazonium tetrafluoroborate,
18: 2-butyl-5-decyloxycarbonylbenzenediazonium tetrafluoroborate,
19: 4-{N-[Y-(2',4'-di-t-amylphenoxy)propyl]carbamoyl}benzenediazonium tetrafluoroborate, and
20: 4-[ß-(2',4'-di-t-amylphenoxy)ethoxy]carbonylbenzenediazonium tetrafluoroborate.

The aryldiazonium salt such as above is generally contained in the reagent layer of the analytical element.

There is no specific limitation of the material constituting the reagent layer employable in the analytical element of the invention, and the reagent layer containing an aryldiazonium salt is prepared by state of the art methods.

However, the reagent layer of the element preferably is in the form of a porous reagent layer so that the diffusion of bilirubin (analyte) can be easily accomplished. The porous reagent layer preferably is in the form of a porous matrix comprising solid fine particles and a binder.

The above-mentioned porous matrix comprising solid fine particles and a binder is formed by porous fine particles or nonporous fine particles such as microcrystalline cellulose, cellulose micropowder, silicate fine particles such as silica, diatomaceous earth, or polymer microbeads, and a binder combining these fine particles to form a porous structure having continuous voids.

As the binder employable for the formation of the porous matrix, a hydrophilic polymer or polymer latex particles containing at least 2% of a hydrophilic repeating unit. Examples of the hydrophilic polymer include homopolymers containing a repeating unit derived from styrene — p-sulfonic acid, acrylic acid, methacrylic acid, a maleic acid derivative, acrylamide, methacrylamide, N-(sulfoalkyl)acrylamide, N-(sulfoalkyl)methacrylamide, N-alkylacrylamide, N-alkylmethacrylamide, N-(hydroxyalkyl)acrylamide, N-vinylpyrroidone, N-vinylimidazole, vinyl alcohol, hydroxyethyl methacrylate and hydroxyethylacrylamide, as well as copolymers containing two or more repeating units derived from the above mentioned repeating units. Copolymers containing the repeating unit derived from the above-mentioned monomer as well as one or more repeating units derived from other monomers can be also employed. Preferably, the binder is an acidic polymer.

The reactivity between bilirubin and a diazonium salt is strongly influenced by the solubility of the bilirubin. Since the free bilirubin (indirect bilirubin) of non-conjugate type is highly hydrophilic and poorly water soluble, the rate of reaction with a diazonium salt is low. In contrast, since the conjugate bilirubin and albumin-conjugated bilirubin are highly soluble in water, these react rapidly with a diazonium salt. Accordingly, the difference of the reaction rate of the variety of bilirubins is utilized to analyze separately

each of the direct bilirubins (conjugate or protein-conjugated bilirubin) and the indirect bilirubin (free bilirubin).

The analytical elements of the present invention can be employed in the same manner to separately analyse each of the direct and indirect bilirubins.

In the quantitative analysis of the total bilirubin content or a quick analysis of the indirect bilirubin, a reaction accelerator is necessarily included in the analytical element. The reaction accelerator employable for this purpose is known and described in a variety of texts. Examples of the accelerators include alcohols (e.g., methanol and ethanol), caffeine, sodium benzoate, sodium acetate, dyphylline (C. A. Registory No. [479—18—5], urea, a nonionic surfactant, gum arabic, an acide amide and sulfoxide. The reaction accelerator can be optionally incorporated into the analytical element of the present invention.

The present invention is further described by the following examples.

Example 1

The aryldiazonium salt defined in the present invention was evaluated in the form of a solution containing it with respect to the function as a bilirubin detection reagent.

Formulation of aryldiazonium salt solution

2-Methoxy-5-(tetradecyloxycarbonyl) benzene diazonium tetrafluoroborate	500 mg
Acetone	15 ml
Ethanol	40 ml

200 µl of this aryldiazonium salt solution was added to 100 µl of each of various control serums in 2 ml of 10% aqueous acetic acid, and the reaction was carried out in the mixture at 25°C for 1 min. Subsequently, the reaction mixture was taken up into a cuvette (lightpath: 10 mm) and the optical density thereof was measured photometrically at a wavelength of 540 nm to determine the content of the produced azobilirubin.

Independently, the total bilirubin content was determined by the use of ABA-200 (available from Abbott Laboratories).

The results are set forth in Table 1.

TABLE 1

	Total Bilirubin Content (mg/dl)	Optical Density (540 nm)
Human albumin solution	0	0.065
Monitrol I (Dade Corp.)	1.8	0.117
Monitrol II (Dade Corp.)	4.3	0.204
Hepatest (Daiichi Seiyaku Co., Ltd., Japan)	10.6	0.395
Versatol P (General Diagonostic Corp.)	18.6	0.683

The relationship between the bilirubin content and the optical density is graphically illustrated in Figure 1. Figure 1 clearly indicates a linear relationship between the bilirubin content and the optical density at 540 nm.

Comparison Example 1

To each 200 ml of the diazonium salt (diazotized sulfanilate) solution attached to a commercially available bilirubin analysis kit and the aryldiazonium salt solution of Example 1 was added each 100 µl of a control serum in 2 ml of 10% aqueous acetic acid. The color reaction was carried out in the mixture at 25°C for 1 min. Subsequently, the reaction mixture was taken up into a cuvette (lightpath: 10 mm) and the absorption spectrum was taken. The absorption spectra given in the use of the diazotized sulfanilate and the aryldiazonium salt are shown in Figure 2.

The absorption spectra shown in Figure 2 indicate that the aryldiazonium salt (Example 1) gives an optical density at a level substantially similar to that of the diazotized sulfanilate (standard reference compound employed in a bilirubin analysis according to the conventional diazo method). Accordingly, it is understood that the aryldiazonium salt has a satisfactory bilirubin detection ability. The absorption spectra further indicate that the spectrum of an azobilirubin produced in the use of the standard reference

compound has the absorption maximum at 520 nm, while the spectrum of an azobilirubin produced in the use of the aryldiazonium salt of Example 1 has the absorption maximum in a wide range of 520 to 550 nm in a longer wavelength region. Accordingly, it is concluded that the aryldiazonium salt of Example 1 is advantageously employed in the analysis of bilirubin.

Example 2

To 10 g of 5% aqueous solution of methyl vinyl ether — maleic anhydride (1:1, molar ratio) copolymer (GANTRETZ AN—139, tradename of GAF Corp., inherent viscosity [η] at 25°C in 1% methyl ethyl ketone solution: 1.0—1.4) was added 5 ml of the aryldiazonium salt solution described in Example 1. To the resulting mixture were added 10 ml of water and 5 g of microcrystalline cellulose (Avicel, trademark) to prepare a coating solution.

The coating solution was coated over a transparent polyethylene terephthalate support to form a layer of 20 µm thick (thickness upon dryness). On this layer was coated 1% aqueous solution of methyl vinyl ether — maleic anhydride (1:1, molar ratio) copolymer, and immediately after the coating was complete, Fuji Microfilter FM 120 (trademark of Fuji Photo Film Co., Ltd., Japan, membrane filter made of cellulose acetate-type blushed polymer, mean pore size 1.2 µm, thickness 180 µm) having been dried after processing with 0.2% aqueous p-nonylphenoxypolyglycidol, was pressed onto the coated copolymer solution. Thus, an integrated analytical element was prepared.

A pure free bilirubin was dissolved in 5% aqueous human albumin solution containing sodium carbonate to prepare indirect bilirubin solutions of three different content levels.

Each bilirubin solution was spotted on the analytical element, and at 4 min. after the spotting, the optical density of color formed on the element was measured by reflection photometry through a green filter. The results are set forth in Table 2.

TABLE 2

Indirect Bilirubin Content (mg/dl)	Δ OD
5	0.23
10	0.32
20	0.50

Remark: "ΔOD" means a value obtained by subtracting from the measured value a value obtained by measurement performed in the same manner except for employing simple 5% aqueous human albumin solution.

Example 3

On a transparent polyethylene terephthalate support was coated 10% aqueous deionized-gelatin solution to form an absorbent layer of 10 µm thick (thickness upon dryness).

Separately, various coating solutions were prepared under the following formulation using various aryldiazonium salts:

Formulation of coating solution for the formation of porous diazonium salt layer

Aryldiazonium salt	40 mg
Diatomaceous earth	10 g
3,3-Dimethylglutaric acid	3 g
5% Aqueous polyacrylamide solution	20 g
Dyphylline	5 g
Water	10 g

The coating solution was coated on the absorbent layer to form a porous diazonium salt layer. After 30 min., a blushed polymer (Fuji Microfilter FM 300: trademark of Fuji Photo Film Co., Ltd., mean pore size 3.0 µm, thickness 180 µm) was pressed onto the diazonium salt layer and then dried to form a porous spreading layer. Thus, a multilayer analytical element was prepared.

On the analytical element was spotted 10 µl of Versatol P (direct bilirubin content 3.8 mg/dl, total bilirubin content 18.6 mg/dl) or Omega (high bilirubin content) standard solution (direct bilirubin content 10.9 mg/dl, total bilirubin content 19.9 mg/dl). The analytical element was then incubated at 30°C for 6 min. and the formed color was measured by reflection photometry at 550 nm. The results are set forth in Table 3.

TABLE 3

Diazonium Salt	Optical Density	
	Versatol P	Omega (high bilirubin content)
2	0.46	0.47
3	0.49	0.50
5	0.48	0.49
6	0.51	0.52
13	0.45	0.46
16	0.47	0.47
17	0.45	0.46
18	0.50	0.51

Remark: The numbers given in Table 3 correspond respectively to the numbers described hereinbefore for listing the representative examples of the aryldiazonium salt according to the present invention. Accordingly, these numbers indicate the following compounds:

2: 2-methoxy-5-(tetradecyloxycarbonyl)benzenediazonium hexafluoroborate,
3: 2-ethoxy-5-(hexadecyloxycarbonyl)benzenediazonium tetrafluoroborate,
5: 2-methoxy-5-[β-(2',4'-di-t-amylphenoxy)ethoxycarbonyl]benzenediazonium tetrafluoroborate,
6: 2-methoxy-5-(N-hexadecylsulfamoyl)benzenediazonium tetrafluoroborate,
13: 4-(hexadecyloxycarbonyl)benzenediazonium tetrafluoroborate,
16: 3-(N-tetradecylcarbamoyl)benzenediazonium tetrafluoroborate,
17: 2-methyl-5-tetradecyloxycarbonylbenzenediazonium tetrafluoroborate, and
18: 2-butyl-5-decyloxycarbonylbenzenediazonium tetrafluoroborate.

Example 4

Preparation of Coating Solution for Formation of Porous Diazonium Salt Layer

In a mixture of 5 ml of ethanol and 2 ml of acetone was dissolved 37.5 mg of 2-methoxy-5-(tetradecyloxycarbonyl)benzenediazonium tetrafluoroborate. The resulting solution was dispersed homogeneously in 10 g of 5% aqueous solution of methyl vinyl ether — maleic anhydride (1:1, molar ratio) copolymer (inherent viscosity [η] in 1% methyl ethyl ketone solution at 25°C: 2.6—3.5). The the resulting dispersion were added successively 30 ml of water, 7.5 g of microcrystalline cellulose (mean particle size 6 µm), and 8 g of dyphylline. The mixture was processed in a ultrasonic dispersing apparatus to give a homogeneous dispersion.

Preparation of Coating Solution for Formation of pH Adjusting Layer

In 30 g of 10% aqueous deionized-gelatin solution was homogeneously dispersed 2.5 ml of divinylbenzene-2-(dimethylamino)ethyl acrylate copolymer latex solution (15%) to prepare the coating solution.

Preparation of Analytical Element for Quantitative Analysis of Bilirubin

On a transparent polyethylene terephthalate support (thickness 180 µm) was coated the coating solution for formation of pH adjusting layer to form a pH adjusting layer of 15 µm thick (thickness upon dryness). On the dried pH adjusting layer was coated the coating solution for formation of porous diazonium salt layer to form a porous diazonium salt layer of 30 µm thick (thickness upon dryness). After approx. 1 min., onto the slightly hardened diazonium salt layer was pressed a cotton cloth under a laminating roller, and the cotton cloth was then dried. Thus, an analytical element for quantitative analysis of bilirubin was prepared.

Analysis of Bilirubin

On the analytical element was spotted 10 µl of a commercially available control. The analytical element was then incubated at 30°C for 6 min. and the formed color was measured by reflection photometry at 550 nm. The results are set forth in Table 4.

TABLE 4

	Total Bilirubin Content (mg/dl)	Optical Density (at 550 nm)
Human albumin solution	0	0.169
Versatol	1.10	0.207
Q-PAK I	1.26	0.215
Calibrate I	1.81	0.211
Monitrol I	1.81	0.224
Calibrate II	3.36	0.304
Monitrol II	4.24	0.321
Q-PAK II	4.77	0.341
Calibrate III	5.07	0.350
Versatol P	18.6	0.644
Omega (high bilirubin content)	19.8	0.675

Remark: The total bilirubin content of each control was quantitatively measured in the manner as described in Example 1 using ABA-200 available from Abbott Laboratories.

Claims

1. An analytical element for quantitative analysis of bilirubin in a liquid sample by diazo method comprising a liquid impermeable, light-transmissive support, a reagent layer containing a bilirubin detection reagent and a liquid sample-spreading layer, characterized in that the reagent layer is a porous reagent layer having continuous voids and the bilirubin detection reagent is a non-diffusive aryl diazonium salt having in the aryl group at least one substituent selected from an alkoxycarbonyl group, an alkylaminosulfonyl group and an alkylaminocarbonyl group.

2. The analytical element claimed in claim 1, in which said aryl group of the aryldiazonium salt further contains at least one substituent selected from an alkyl group and an alkoxy group.

3. The analytical element claimed in claim 1, in which the porous reagent layer has a porous matrix comprising solid fine particles and a binder.

4. The analytical element claimed in claim 1, in which the reagent layer comprises an acidic polymer.

Patentansprüche

1. Analytisches Element zur quantitativen Analyse von Bilirubin in einer flüssigen Probe nach der Diazomethode, enthaltend einen flüssigkeitsundurchlässigen, lichtdurchlässigen Träger, eine Reagensschicht, die ein Bilirubin-Erfassungsreagens enthält, und eine Ausbreitungsschicht für die Flüssigkeitsschicht, dadurch gekennzeichnet, daß die Reagensschicht eine poröse Reagensschicht ist, die kontinuierliche Hohlräume enthält, und daß das Bilirubin-Erfassungsreagens ein nichtdiffundierbares Aryldiazoniumsalz ist, welches in der Arylgruppe mindestens einen Substituenten, ausgewählt aus einer Alkoxycarbonylgruppe, einer Alkylaminosulfonylgruppe und einer Alkylaminocarbonylgruppe, aufweist.

2. Analytisches Element nach Anspruch 1, dadurch gekennzeichnet, daß die genannte Arylgruppe des Aryldiazoniumsalzes weiterhin mindestens einen Substituenten, ausgewählt aus einer Alkylgruppe und einer Alkoxygruppe, enthält.

3. Analytisches Element nach Anspruch 1, dadurch gekennzeichnet, daß die poröse Reagensschicht eine poröse Matrix aufweist, die feste feine Teilchen und ein Bindemittel enthält.

4. Analytisches Element nach Anspruch 1, dadurch gekennzeichnet, daß die Reagensschicht ein saures Polymeres umfaßt.

Revendications

1. Elément d'analyse pour la détermination quantitative de la bilirubine dans un échantillon liquide par procédé diazo, comprenant un support translucide, imperméable aux liquides, une couche de réactif contenant un réactif de décèlement de bilirubine et une couche de dispersion d'échantillon liquide, caractérisé en ce que la couche de réactif est une couche poreuse de réactif présentant des interstices continus et le réactif de décèlement de bilirubine est un sel d'aryldiazonium non diffusant qui contient dans le groupe aryle au moins un substituant choisi parmi un groupe alcoxycarbonyle, un groupe alkylaminosulfonyle ou un groupe alkylaminocarbonyle.

2. Elément d'analyse selon la revendication 1, dans lequel ledit groupe aryle du sel d'aryldiazonium contient en outre au moins un substituant choisi parmi un groupe alkyle ou un groupe alcoxy.

3. Elément d'analyse selon la revendication 1, dans lequel la couche poreuse de réactif présente une matrice poreuse comprenant de fines particules solides et un agent liant.

4. Elément d'analyse selon la revendication 1, dans lequel la couche de réactif contient un polymère acide.

FIG.1

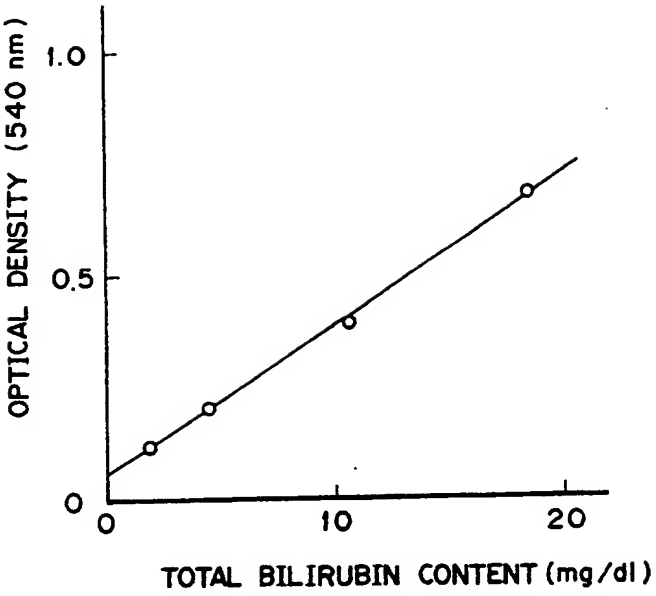
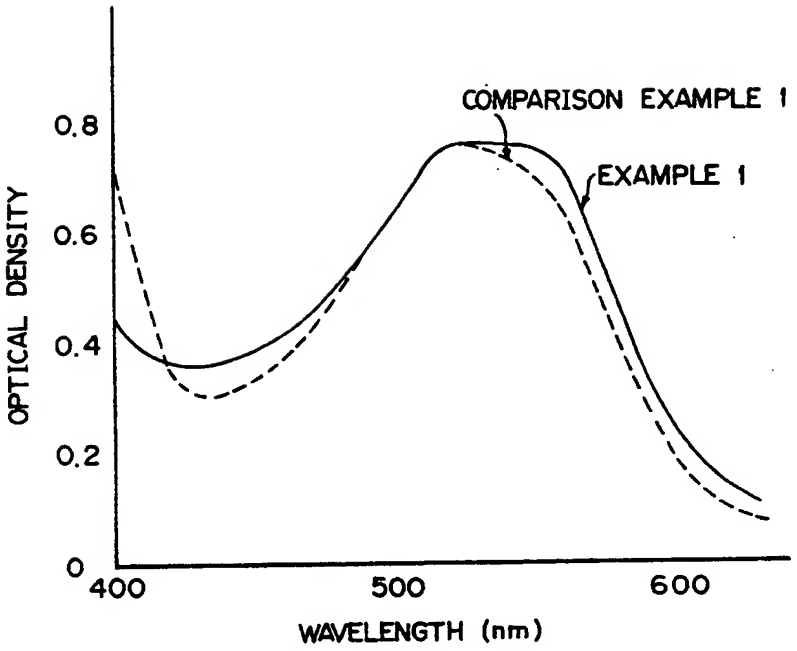

OPTICAL DENSITY (540 nm)
1.0
0.5
0
0
10
20
TOTAL BILIRUBIN CONTENT (mg/dl)

FIG.2


COMPARISON EXAMPLE 1
EXAMPLE 1
OPTICAL DENSITY
0.8
0.6
0.4
0.2
0
400
500
600
WAVELENGTH (nm)